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A Functional Analysis of Circadian Pacemakers in Nocturnal Rodents

V. Pacemaker Structure: A Clock for All Seasons

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Summary. 1. This paper is an attempt to integrate in a general model the major findings reported earlier in this series on: lability and history dependence of circadian period, τ (Pittendrigh and Daan, 1976a); dependence of τ and α on light intensity as described in Aschoff's Rule (Daan and Pittendrigh, 1976b); the interrelationships between τ and phase response curves (Daan and Pittendrigh, 1976a); and those inconsistencies between experimental facts on entrainment and theoretical predictions based on a single oscillator with fixed parameters τ and PRC, which pointed to a more complex system (Pittendrigh and Daan, 1976b).

2. The qualitative model developed consists of two oscillators. The evidence that two separate oscillators are involved in circadian activity rhythms rests largely on the "splitting" phenomenon, known to occur in several species of mammals and birds.

3. The empirical regularities of "splitting" in hamsters exposed to constant illumination (LL) are described:

(i) Splitting, i.e. the dissociation of a single activity band into two components which become stably coupled in circa 180° antiphase, occurs in about 50% of the animals in 100–200 lux, and has not been observed in lower light intensities.

(ii) Splitting never occurred before 40 days after the transition to LL, unless the pretreatment had been LL of low intensity. In some animals the unsplit condition returned spontaneously.

(iii) The attainment of antiphase is usually accompanied by a decrease in τ, and refusion of the two components by an increase in τ. These data show that both the split and the unsplit condition are metastable states, characterized by different phase relationships (ψ_{EM}) of two constituent oscillators. ψ_{EM} is history-dependent and determines τ of the coupled system.

4. Observations in *Peromyscus leucopus* transferred from LL to DD to LD 12:12 show that the two components of the bimodal activity peak (in LD)

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can for some time run at different frequencies (in DD), suggesting that bimodality of activity peaks and splitting are based on the same two-oscillator system.

5. The model developed assumes the existence of two oscillators or principal groups of oscillators E and M, with opposite dependence of spontaneous frequency on light intensity. The dependence of the phase relationship (ψ_{EM}) between the two on light intensity and the dependence of τ on ψ_{EM} account for all the history-dependent characteristics of circadian pacemakers, and for the interdependence of τ, PRC, and τ-lability. The model qualitatively accommodates the interdependence of τ and α summarized in Aschoff's Rule. It is noted that the major intuitive elements in the model have been found to characterize an explicit version of it in computer simulations. The relevance of the model to seasonal change in photoperiod is discussed. A pacemaker comprising two oscillators mutually interacting but coupled separately to sunrise and sunset enhances its competence to accommodate to seasonal change in the daily pattern of external conditions; and it could well be involved in the pacemaker's known ability to discriminate between daylengths in the phenomena of photoperiodic induction.

I. Introduction

The previous paper concluded that the principal empirical generalizations about circadian pacemakers reflect their clock-like function. But precisely because the time-measuring functions they reflect must everywhere be met in unicellulars, plants and animals alike, none of them provides any assurance that a common mechanism is involved; none is, therefore, a guide to concrete mechanism. There may, of course, be some evolutionary conservatism involved; but evolutionary convergence is at least as likely a cause. Brinkmann (1971) has already given reason to believe that the temperature-compensation of τ (a special case of its more general homeostasis) may be effected by different mechanisms even in the same cell (*Euglena gracilis*) in different metabolic states.

Winfree (1975a) has recently chosen to emphasize the "Unclock-like Behaviour of Biological Clocks." However, the phenomenon that continues to hold his attention (e.g., Winfree, 1970a, b, 1971, 1973a, b, c, d, 1975a, b), though elegant in conception, is itself no clue to the physical structure of circadian pacemakers; and its irrelevance to their function is stressed by Winfree himself in the title of his paper. He has accomplished the delicate feat of "stopping" the *D. pseudoobscura* pacemaker by driving it onto the "singularity" of its phase-plane where it is, apparently, stably motionless (Winfree, 1973d). He has done the same to oscillations in the redox state of NAD which he generates by disturbing the steady-state of yeast glycolysis (Winfree, 1975b).

The behavior common to these systems is obviously not the result of evolutionary convergence in the usual functional sense. But that is not the only hazard in seeking clues to concrete mechanism. In all oscillating systems capable of self-sustained "limit cycle" movements, no matter what their physical nature, there is, as analytic necessity, a point equilibrium or singular state on the phase-plane

(see, e.g., Pavlidis, 1973). The only empirical question is whether or not that singularity is stable – and how stable. Even when it is apparently stable, very different concrete causes may be responsible. Topological isomorphisms in complex biological systems provide no surer guide to physical mechanism than functional isomorphisms.

The only general measures of a model's merit, other than taste, are (i) its adequacy to account for the facts it addresses and (ii) its utility in promoting analytical progress. In the life sciences the latter may take either of two directions – the analysis of functional organization or of concrete physical mechanism – which in the long run hopefully converge. The model of pacemaker structure sketched in this paper must be judged by both criteria. It was initially conceived to explain the facts we describe as "splitting" and "refusion" of the daily activity period (Pittendrigh, 1974), but it also provides a basis for understanding other aspects of circadian pacemakers that are among their most characteristic features and not addressed by any other model. We note that several authors have previously proposed, in more general terms, a system of two coupled oscillators as responsible for various other circadian phenomena [Pittendrigh, 1960; Engelmann, 1966; Gwinner (in Aschoff, 1967); Hoshizaki et al., 1974].

For methods used in obtaining the activity records discussed in this paper we refer to the first article in this series (Pittendrigh and Daan, 1976a).

II. The "Splitting" Phenomena

"Splitting" was first encountered as a response of the arctic ground squirrel (*Spermophilus undulatus*) freerunning in constant light (Pittendrigh, 1960; Fig. 11). Following an abrupt increase in light intensity, a component in the animal's activity that originally occurred at the beginning of its subjective day began to recur at increasingly later times until after about 50 cycles it became a wholly distinct band of activity scanning across the (diurnal) animal's subjective night. After ca. 100 cycles it eventually regained its original phase-relation with the rest of the animal's activity. There was some indication that – associated with the split – the period of the animal's major activity band was shortened.

The behavior of that one squirrel was subsequently reported to be a reproducible phenomenon in the golden hamster, *Mesocricetus auratus* (Pittendrigh, 1967). In this case it was always a component of activity in the animal's late subjective night that recurred at a higher frequency (occurring at increasingly earlier times) than the rest. Hoffmann (1970, 1971) then reported that the splitting phenomenon was even more reproducible in the small diurnal primate (tree shrew) *Tupaja belangeri*. In *Tupaja* it is a drop in light intensity that induces splitting. Table 1 lists the reported cases of splitting suggesting the behavior may be widespread.

Aschoff (1954, 1957) noted many years ago that the daily activity pattern of vertebrates commonly includes two distinct components. These are often clear even in event-recorder data of the type we are analyzing, but such recordings sometimes fail to make the two peaks clear when the animal is very active throughout its activity time (α) (cf. Fig. 1). In what follows we shall designate the first major peak of activity which occurs in the early subjective night (beginning of α)

Table 1. Occurrence of "splitting" into two components of circadian activity rhythms

Species	Day- or night-active	Splitting induced by	Reference
Tupaja belangeri	day	light intensity decrease	Hoffmann, 1971
Spermophilus undulatus	day	light intensity increase	Pittendrigh, 1960
Funambulus palmarum	day	light intensity decrease	Pohl, 1972
Glaucomys volans	night	light intensity increase	Daan, unpublished
Mesocricetus auratus	night	high light intensity	Pittendrigh, 1967, 1974
Mesocricetus auratus	night	hypothalamic lesions	Rusak and Zucker, 1975
Sturnus vulgaris	day	testosterone implants	Gwinner, 1974

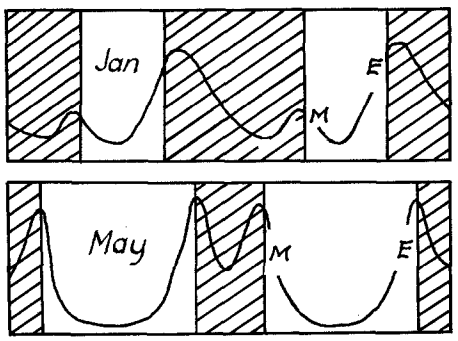

Fig. 1. The bimodal distribution of activity in *Mus musculus* under two different photoperiods. *E* and *M* designate the evening (*E*) and morning (*M*) peaks discussed in the text. Based on data given by Aschoff (1954)

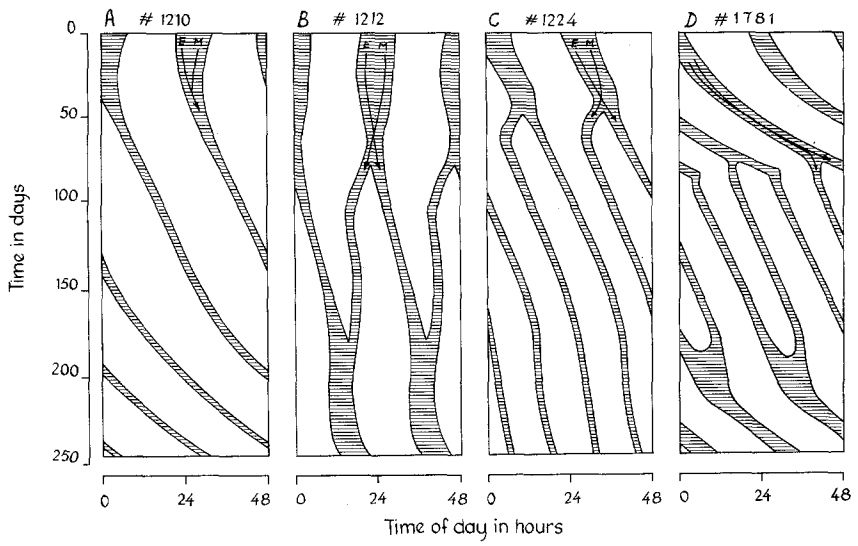

Fig. 2 A–D. The types of response of hamsters *(M. auratus)* to constant light (100–200 Lux). (A) The rhythm remains "unsplit", and τ continues to lengthen as a function of time. The activity band (α), comprising *E* and *M* components, is reduced initially as τ lengthens. (B) τ fails to lengthen; eventually the *M* component breaks free and, running at a higher frequency than *E*, scans the entire cycle until it regains its original phase relative to *E*. (C) *M* again breaks free and runs at a higher frequency than *E*, but its advance is arrested when it phase-leads *M* by $\sim 180°$. The system remains stably split and its τ gradually shortens as a function of time. (D) τ lengthens and α remains unsplit for ~ 60 cycles until *M* breaks free; *E* continues with its former long τ until the 180° antiphase is reached when τ of the split system abruptly shortens. The two components spontaneously "refuse" ($\sim$ day 175) and the unsplit system resumes its characteristially long τ

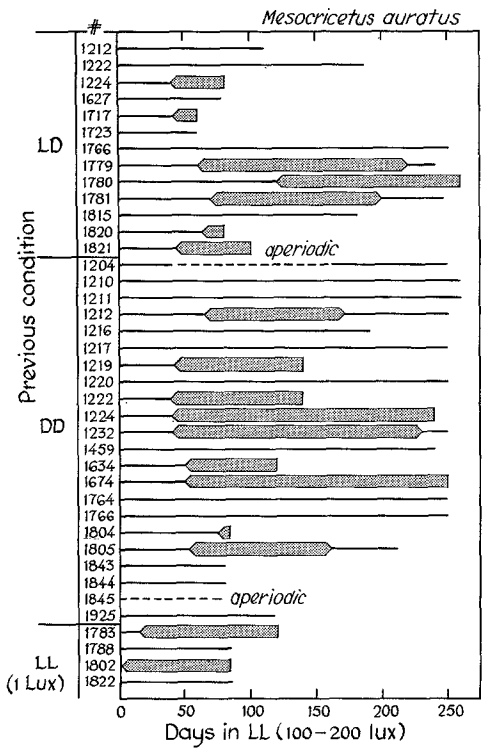

Fig. 3. The time course of splitting of hamster activity rhythms in LL. Each animal is indicated by a horizontal line. Four animals (# 1212, 1222, 1224, 1766) were studied twice in LL. Shaded areas indicate the time during which the rhythm was split into two components. Splitting occurred in 18 out of 39 cases. In 5, the two components re-fused again before the LL-condition was terminated. Two animals were aperiodic (dashed lines). Previous conditions did not seem to affect the frequency of splitting, although both animals splitting after LL (1 Lux) pretreatment did so within 30 days; in other animals the phenomenon was never observed before 40 days after the beginning of LL

as *E* (evening) and the later component that precedes dawn is designated *M* (morning).

Figure 2 summarizes schematically the range of responses we have encountered when hamsters are transferred either from DD, LD *12*:12 or low intensity LL to continuous light of between 100 and 200 Lux. Figure 3 gives, again schematically, a summary of how all individuals studied behaved in the course of many months exposure to LL.

In slightly less than 50% of the animals the rhythm continues to involve a single activity band (α) in each circadian cycle: α is small and τ, which is longer than the period found in DD often continues to lengthen as a function of time – even for over 150 cycles (Fig. 2A), although steady-state τ-values on the average are reached in about 70 days (Daan and Pittendrigh, 1976b, Fig. 7). In a minority of cases (2 out of 39 recorded) the animal rapidly became completely aperiodic; frequent short bursts of activity revealed no circadian periodicity. In one of these cases, however, (Fig. 4) these sporadic bursts began to "nucleate" after approximately 5 months in LL and a clear circadian rhythm developed. Its period (τ)

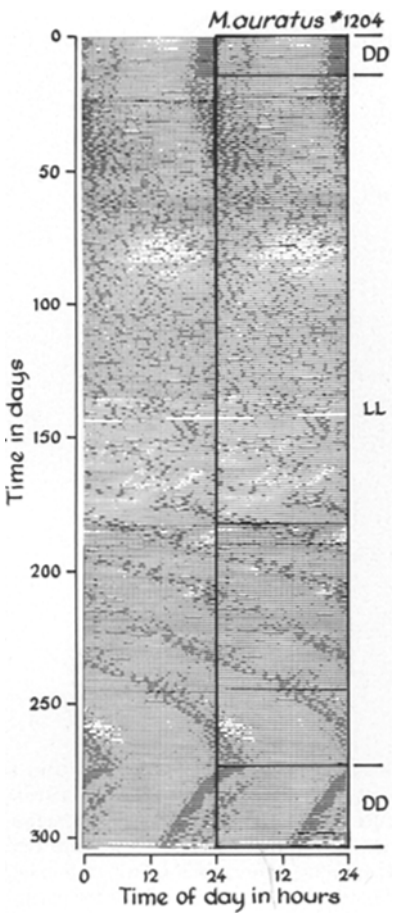

Fig. 4. Activity record of a hamster developing aperiodic activity in LL (100–200 Lux) and regaining a circadian rhythm after ca. 150 days. 2 of 39 animals exposed to LL (see Fig. 3) were aperiodic

was greater than any other encountered in a hamster but became progressively shorter with time.

In 18 out of 39 of the animals splitting occurred. Sample raw data illustrating the phenomenon are given in Figure 5 (see also Pittendrigh, 1974, Fig. 11). In several cases where the split develops gradually it is clear that the activity (M) which occurs late in the subjective night (end of α) breaks away and phase advances relative to the E component. In one case (Fig. 2B) this phase-advance of M continued across the animal's entire subjective day until it regained its original phase-relation to E. Then its motion stopped and the system remained stable. A comparable but more rapid scan of the entire cycle is seen in Figure 5 (#1717). This behavior (Fig. 2B) is strictly comparable to that of *Spermophilus* (Pittendrigh, 1960, Fig. 11), except that the dissociating component phase-delayed in the squirrel case.

The behavior recorded by Figure 2B and Figure 5 (#1717) appears to be unusual in hamsters; the gradual phase-advance of M is usually arrested, quite abruptly, when it has reached a 180° antiphase relative to E (Figs. 2C and 2D).

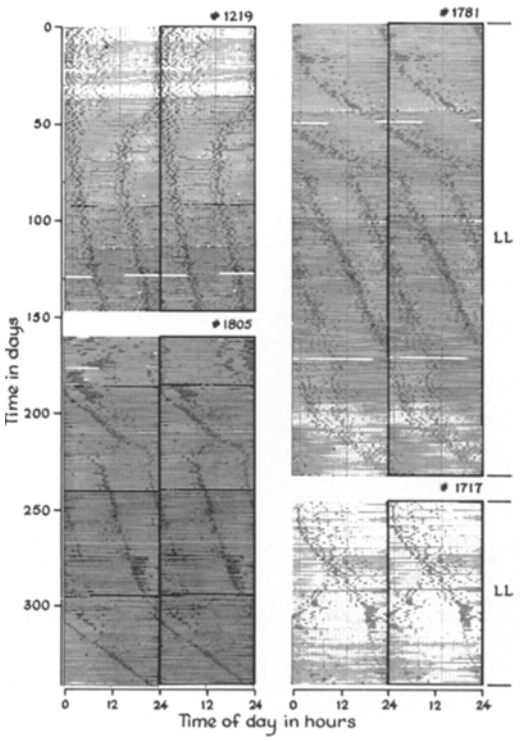

Fig. 5. Examples of "splitting" of the activity rhythm in hamsters due to exposure to constant light LL (100–200 Lux). Notice that (1) splitting does not start immediately after release into LL, but rather after 40–70 days, (2) when τ_{LL} is long (#1781, 1805, 1717), it becomes conspicuously shorter by splitting, and lengthens again when the two components merge together (#1781, 1805). Other examples have been published by Pittendrigh (1967, 1974)

M and *E* then remain as two clearly distinct bursts of activity in each circadian cycle lying about 180° relative to each other. This phase-relation is typically stable: in the animal whose activity pattern is schematized in Figure 2C the new phase-relations between *M* and *E* persisted for nearly a year.

The majority of splits develop so abruptly that it is not possible to identify with certainty which of the split components is *M* and which is *E*. The abrupt cases, however, draw attention to a feature that probably characterizes all split systems. When abrupt splits occur there is an equally sudden change in the period of the whole system. Prior to the split τ is long (as it is in most LL animals that never split, Fig. 2A) but promptly shortens when the split occurs. In fact, the data commonly permit a more restrictive statement: τ shortens when the antiphase is reached and the new steady-state phase-relation between *M* and *E* is established. This is clear in Figure 2D and Figure 5 (#1805, #1781, and #1717; see also Fig. 6).

Figure 2D and animals #1805 and #1781 in Figure 5 illustrate another common aspect of LL induced behavior: the split system is prone to a spon-

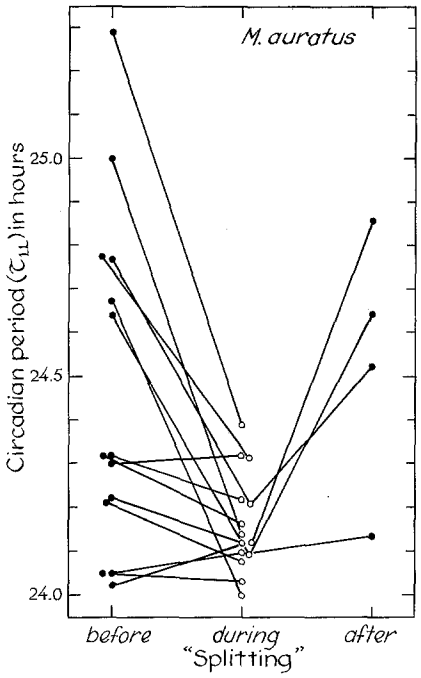

Fig. 6. Changes in τ related to splitting into two components of the activity rhythm in LL in golden hamsters, and re-fusing of the two components in four of the animals

taneous "refusion"; and when the *E* and *M* components regain their former phase relation, the unsplit system reverts to its characteristic longer τ (Figs. 5 and 6).

There is a suggestion in our data that when splitting proceeds gradually (Fig. 2B and 2C and #1219 in Fig. 5) the long τ characteristic of other unsplit systems was never realized in LL. And while splitting usually imposes a change in τ of both components (Fig. 2D and all other abrupt cases) sometimes *M* appears to have little impact on *E* when the antiphase is reached and entirely assumes *E*'s own period (Fig. 2C).

Several general statements are warranted by the data:

1. Splitting is usually slow to develop in LL; the unsplit system is usually "stable" for about two months. The majority of splits occur between 40 and 60 days after the beginning of LL (Fig. 3).

2. Splitting requires a minimum light intensity: It was never seen in LL 1 Lux, but when transferred from this dim illumination to 100–200 Lux, splits developed rapidly.

3. Splitting is often accompanied by a change in τ of the entire system; the shortening of τ is evident only when the 180° phase-relation between the two components is completed.

4. Refusion of the split system is usually accompanied by a return to long τ.

We have not encountered the splitting phenomenon, induced by high intensity LL, in any of our other nocturnal rodents. On the other hand it is often very clear that when *Peromyscus leucopus*, characterized by small α in LL, is transferred to constant dark the resulting gradual increase in α is due to the dissociation

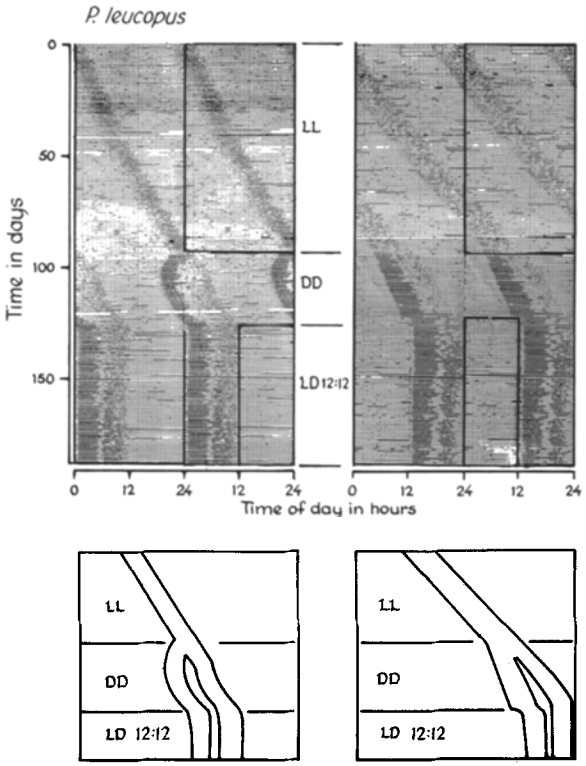

Fig. 7. The dissociation of two components (*E* and *M*) in the activity band of *Peromyscus leucopus* on entry in DD from LL. (Left, animal # 1445; right, # 1442). Raw data above; interpretation below. See text

of two distinct components. The two cases in Figure 7 leave no doubt about the different behavior of these components while α expands. One of them continues with the long τ established in the preceding LL; its period shortens only when the other component, moving at a high frequency, establishes the phase-relation between them that is characteristic of DD: only then is the period (τ) characteristic of DD expressed by both components. What these records show convincingly is that the bimodal pattern of activity in LD 12:12 is indeed produced by the same two components diverging during the DD-interval from their prior compressed state in LL.

III. Pacemaker Structure: Mutually Coupled Oscillators?

a) Two Stable Steady States of the Freerunning Pacemaker

The phenomena of splitting and refusion challenge any pacemaker model based on a single oscillator (Pittendrigh, 1960; Hoffmann, 1970). On the other hand several of the strongest features seem readily explained as the behavior of two

mutually coupled oscillators (Pittendrigh, 1974). In this model one oscillator (M) is taken to control the morning component of α; the other (E) controls the evening component. We designate their theoretical intrinsic periods, i.e. the periods they would display if they could be uncoupled, as τ_M and τ_E respectively.

There are four propositions in the model: 1. the intrinsic period of both oscillators depends differently on light intensity: τ_E is a positive and τ_M a negative function of intensity; 2. when mutually coupled, i.e. when synchronizing each other, their interaction results in the compound pacemaker expressing a period τ, different from the period of either of its components; 3. the relative influence of each component on the other depends, among other things, on the phase-angle difference (ψ_{EM}) between them; 4. in some circumstances, such as constant light of high intensity, two distinct coupling-modes (CM-1, CM-2) are possible; one of these (CM-1) corresponds with the unsplit, and the other (CM-2) with the stably split system.

The central features of the model are propositions 2 and 3: the period of the system depends on the interaction of the two components, and the strength of the interaction depends on their mutual phase-relation (ψ_{EM}). Both propositions are exemplified in the abruptness with which the coupled system's period shortens when its constituents reach the 180° antiphase: in Figure 2D and Figure 5 (#1805 and #1781) the component we take to be E maintains its previous long period *until* M has reached the 180° antiphase when the periods of both components change.

As in other nocturnal rodents the period of the hamster's freerunning pacemaker lengthens when the animal is transferred from darkness (DD) to constant light (LL). Although the change in period caused by light is unusually small in *M. auratus*, it increases, as in other rodents, when the intensity is increased (Daan and Pittendrigh, 1976b). We have no evidence that splitting occurs at intensities lower than ~100 Lux. Even between 100 and 200 Lux it occurs in only 50% of the animals and here it takes typically about 60 cycles before the split occurs. Thus, even in a given environment two distinct states are realizable: unsplit and split. We attribute this, in our model, to the existence of two distinct coupling modes: CM-1 (unsplit) and CM-2 (split). Both states are metastable: CM-1 may persist for 60 cycles under conditions in which CM-2 is equally stable; the compound system is prone to revert spontaneously from CM-2 to CM-1. The phase-angle difference (about 180°) between E and M in CM-2 is such that the system's period is shorter than it is in CM-1 where the phase-relation between the mutually interacting components is very different.

b) A Structural Basis for Aschoff's Rule

The compound pacemaker's period is dependent on light intensity even when it remains in the unsplit state (CM-1). This is explained, qualitatively, if we assume that τ_E is a positive and τ_M is a negative function of light intensity. When these intrinsic periods of the constituent oscillators are changed by change in light intensity the period of the coupled system will also change; so will the phase-angle difference (ψ_{EM}) change. The interdependence of τ and α change induced

by light intensity (Aschoff's Rule) is readily explained in this way, since in the model α reflects the phase relationship between the two constituent oscillators. Whether the change in pacemaker period induced by constant light is positive, as is usual in nocturnal forms, or negative (diurnal animals), will be determined by the strength with which the two oscillators influence each other.

The period of the unsplit system in hamsters increases with light intensity as long as the normal coupling-mode (CM-1) persists. Splits develop when that normal pattern of interaction fails: M then temporarily escapes from entrainment by E and freeruns until it is usually (Fig. 2B is an exception) recaptured by E in a distinct coupling-mode in which the oscillators again interact, but in such a way that the system's period is different.

c) History-Dependence of Pacemaker States; Hysteresis

The difference in the pacemaker's period and the phase-relationship between its constituent oscillators (ψ_{EM}) which characterize the split and unsplit states imply that the strength of $E-M$ interactions which govern τ in LL depends on ψ_{EM}. That relationship provides a basis for the strong history-dependence of the freerunning pacemaker's period and activity time (α), also when it remains in the unsplit coupling mode.

There must be some most probable state of the system, characterized by a particular τ_p and ψ_{EM} for any given light intensity that will depend primarily on the values of τ_E and τ_M of the uncoupled components at that light intensity, including darkness. However, the actual state of the system at any given time may not be its most probable for that environment: prior conditions will have imposed a particular ψ_{EM} which so affects $E-M$ interaction that the most probable state for the environment may not be rapidly realized. In short, the phase-relation (ψ_{EM}) between the system's component oscillators, established "historically", could account for one of the most characteristic properties of circadian pacemakers which is their susceptibility to "after-effects" on both τ and α (Pittendrigh and Daan, 1976a).

We also see here one potential reason why high light intensity fails to induce splitting immediately – and sometimes not at all. The intrinsic periods (τ_E and τ_M) of the pacemaker's components may be changed immediately but the strength of their interaction will remain influenced by their phase relation (ψ_{EM}) which was established in the previous environment. In short, initial conditions again play a role in determining which of two possible steady-states are realized. It is not unlikely in a system where two stable equilibria occur, that slight changes in any of the parameters involved may render either of the two equilibria unstable and cause the system to move to the other stability. It is a remarkable coincidence that most splits in hamsters developed after 40–60 days in LL (Fig. 3). Possibly, testicular recrudescence, which involves similar time constants in LL, by modifying the endocrine balance, contributed to the incidence of splitting. There is a clear precedent in the induction of splitting in starlings by testosterone implantation (Gwinner, 1974). Testosterone is further known to affect parameters of the circadian activity rhythm in mice in a way which suggests differential effects on morning and evening components (Daan et al., 1975).

The few incidences of fusion of the split components, which are less regularly distributed in time, can also tentatively be explained by a change in system parameters. While the estimate of 100–200 Lux allows for a broad range of light intensities measured in different parts of the cages and at different times of the experiment, it is not excluded that in some cabinets a gradual decrease in light intensity due to aging of the lights in the course of these long experiments has escaped our notice. Such a decline would be expected to help restore the unsplit condition.

The hysteresis Hoffmann (1971) encountered in *Tupaja* is also interpretable in terms of two stable equilibria. He found that after the tree shrew's activity rhythm had split – for which a particular low light intensity was necessary – it remained split even when the illumination was raised above the intensity that initially induced the split. Much higher light intensities were necessary to force the system back into the unsplit state. Here again the state of the system depends not only on the τ that each component would express, were it freerunning at the prevailing light intensity but on the strength of their mutual interaction which also depends on the then-prevailing phase-relation between them – and that is history-dependent.

d) The Interdependence of τ, α and PRC

Our interpretation of the history-dependence of pacemaker state provides a qualitative explanation of all after-effects and the framework for understanding some of the more specific features of pacemaker lability and the interdependence of its major parameters.

The model implies that the pacemaker's measured phase-response-curve (PRC) reflects the net phase-shifts ($\Delta\phi$) of the coupled system pulsed at different phases of its cycle. The $\Delta\phi$ elicited at any phase (ϕ) depends not only on the response of each oscillator, separately, to the light pulse, but on their mutual interaction following their separate $\Delta\phi$'s. Here, especially, the range of possibilities – unconstrained by present data – makes detailed elaboration pointless. There is, however, one aspect which we judge is largely independent of detail and relevant to one of the more remarkable pacemaker properties reported earlier (Daan and Pittendrigh, 1976a): the shape of its PRC is correlated with change in the compound pacemaker's period (τ). Change in τ of the compound pacemaker is supposedly accompanied by a change in ψ_{EM}, the phase-angle difference between its compound oscillators; the strength of their mutual interaction depends on ψ_{EM}; and since the *net* phase-shift ($\Delta\phi$) of the system pulsed at any ϕ must also be influenced by the strength of $E-M$ interaction, the net $\Delta\phi$ elicited at any ϕ will change as τ changes.

There is no indication in the available data pointing to a specific shape of the phase response curves for brief light pulses of each oscillator separately. Both may comprise a "complete" PRC, with phase advances and phase delays. Alternatively, one might presume that the PRC of oscillator E has only phase delays (in correspondence with the assumed increasing effect of light on τ_E) while the PRC of oscillator M has only phase advances. In the latter case, we should expect

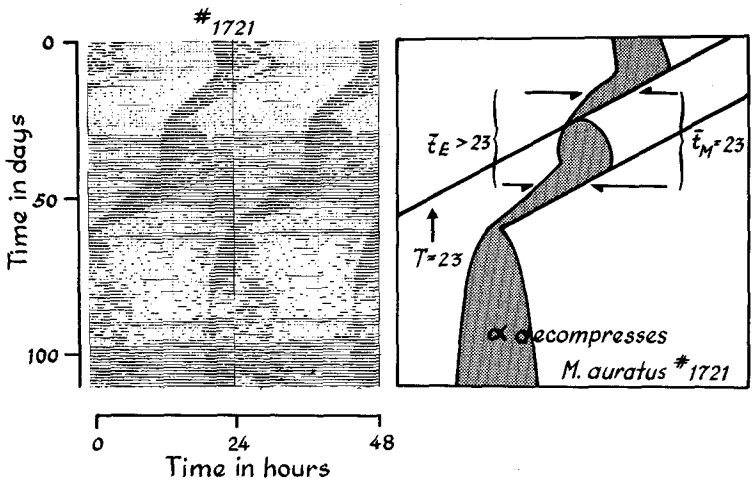

Fig. 8. The compression and decompression of α in *Mesocricetus auratus* during "relative coordination" in a 23 h light cycle (one 15′ pulse/cycle); left panel: raw data; right panel: summary; $\bar{t}_E$ is the average interval between activity onsets; $\bar{t}_M$ average interval between activity cutoffs

that, when exposed to a 24 h light cycle the entrained steady-state of the compound pacemaker depends on the interaction of a phase-advance exerted on M by the morning signal and a phase-delay on E by the evening signal. Thus the state of the freerunning pacemaker, characterized by ψ_{EM}, will not only be changed by entrainment and show after-effects on τ and α on subsequent release, but change systematically as the interval (photoperiod) between the morning and evening pulses is increased: we should then, given its history-dependence, expect to find after-effects not only of T (the period of the light cycle) but also of its photoperiod. The most powerful argument for this interpretation emerges from the after-effects of skeleton photoperiods in *Mus musculus*. Two 1 h pulses, separated alternatingly by 7 and 15 h of darkness produced significantly different τ's in subsequent DD-freeruns when mice had had their activity compressed in the short interval, or expanded in the long interval (Pittendrigh and Daan, 1976a, Figs. 14, 15). Apparently, it is not the characteristic of the light-dark cycle itself, but how it has modified, in the two possible ways, the internal phase-relationships in the animal, that led to the τ subsequently expressed in DD.

When the hamster's compound pacemaker fails to entrain to cycles of, e.g., 23 h there are conspicuous features of its behavior that are open to explanation in terms of our model. Initially, the end of the activity-band (α) which is controlled by the M oscillator, entrains well to the light pulse in each cycle: it responds with the daily advance phase shift of circa one hour necessary to follow the light (Fig. 8). The light also has a clear influence on the behavior of the E-oscillator: activity onsets (under the control of E) are driven at a shorter period than when the system freeruns. But the impact on M and E is inadequate to maintain the compound system at a period of 23 h; thus the period between activity onsets is never reduced completely to 23 h with the result that α is steadily compressed to smaller and smaller durations (ψ_{EM} is reduced). Eventually M fails entirely to control E and

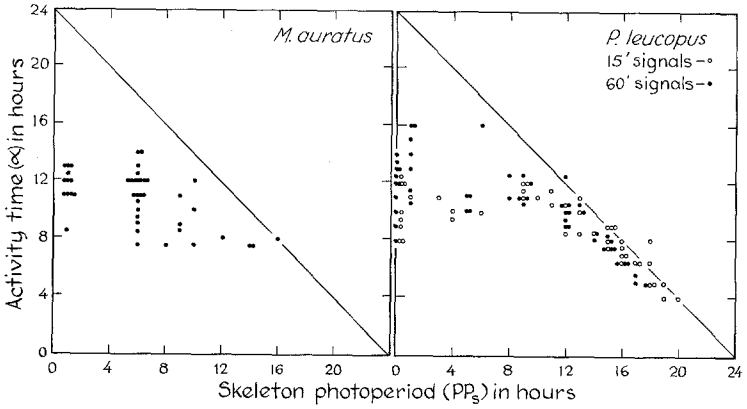

Fig. 9. The compressibility of α by skeleton photoperiods in hamsters (left) and white-footed deermice (right). PPs = the interval complementary to the interval during which most activity occurred. At PPs = 0, α-values for the same animals in DD are shown, at PPs = 0.15 (○) α-values in T 24 (one 15′ pulse per cycle), at PPs = 1 (●) α-values in T 24 (one 60′ pulse per cycle). In hamsters α can be compressed only to ca 8 hours by a 16 hr skeleton photoperiod, beyond which phase jumps occur. In *P. leucopus* α can be compressed down to 4 h by a 20 h skeleton photoperiod. Based on the experiments reported in Fig. 15 and 16 of Pittendrigh and Daan 1976 b

the coupled system breaks away from the light pulse when α promptly begins to decompress as M and E slowly return to their mutual phase-relation characteristic of the freerunning system. But that return is slow: if the light pulse is discontinued α gradually expands to its normal DD-value of ca. 10 h.

The compression of α due to change in internal phase relationships now also explains why the model of nonparametric entrainment, which to some extent accurately relates steady state entrainment by short light pulses to parameters of the freerunning pacemakers (τ and PRC), failed in the skeleton photoperiods beyond ca. 12 h (Pittendrigh and Daan, 1976 b). In *Peromyscus leucopus*, the activity time could be compressed between two light pulses down to ca. 4 h, about half of its normal DD-value (Fig. 9). Not only must this change in ψ_{EM} have caused the severe distortion of the phase response curve, but also a major change in τ. As originally formulated, based on rigidly fixed PRC and τ, the model clearly is wrong. But it has served its best possible function by leading to the experiments, which showed where it was wrong, and precisely for which reasons.

e) The Tightness of Homeostatic Control: Species Diversity

Throughout these papers (Pittendrigh and Daan, 1976 a, b; Daan and Pittendrigh, 1976 a, b) we have used the results from different species to illustrate different phenomena. This is no accident. While in the early stages of the work the choice of experimental animals was to some extent haphazard, in the later stages we became aware of the considerable differences between species. Table 2 is an attempt to summarize these differences. It contains some gaps, arbitrariness and subjective judgement, since all animals were not necessarily subjected to the same

Table 2. Species difference in pacemaker properties. Subjective indications are used: 0=none, +=small, ++=medium, +++=large, blank=no information available

Species	*M. auratus*	*P. leucopus*	*M. musculus*	*P. maniculatus*
Overt rhythm:				
day-to-day variation	+	++	++	+++
Freerunning period:				
departure of $\bar{\tau}$ from 24.0 h	0	0	++	+++
intra-individual variation	+	+	++	+++
inter-individual variation	+	+	++	+++
change in $\bar{\tau}$ with age	+	+	++	+++
change in $\bar{\tau}$ by light (LL)	+	++	+++	+++
change in $\bar{\tau}$ by D_2O	+	++	+++	++
after-effects of LL	0	+		
after-effects of photoperiod	0	+	+	
after-effects of $\Delta\phi$	+	+	+	+
after-effects of T	+		+++	
Activity time (α)				
duration α	+	++	+++	+++
intra-individual variation	++	++		+++
inter-individual variation	++	++		+++
change in α with age	+			+
change in α by light (LL)	+			+
incidence of splitting	++	0	0	0
compression of α by PPs	+	++		
Phase-response curves (PRC)				
delay part D	+	+	+++	+++
advance part A	+++	+	+	+

experimental protocol. But even with this reservation the species comparison reveals a clear correlation between a number of pacemaker properties. The hamster differs from the other species by being less subject to various kinds of pacemaker variability and lability. This holds for precision of the overt activity rhythm as well as for the inter- and intraindividual variation of τ, whether "spontaneous" or attributable to such causes as: age, prior conditions ("after-effects"), intensity of constant illumination. At the other end of the species sequence we find both *Mus musculus* and *Peromyscus maniculatus*, where τ is labile within the individuals, and variable between them. Is it by accident that splitting occurs in hamsters only? We think not. But the correlation does not pinpoint any specific difference, even in a deterministic two-oscillator model (Daan and Berde, in preparation). Does the tighter homeostatic control in hamsters imply that their two oscillators are more nearly equal in parameters, which would lead not only to more rigid fixation of frequency, but also to the existence of two equally stable coupling equilibria, while in *Mus* and *Peromyscus* increased dominance of one (E) over the other (M) oscillator would cause greater variability and lability, e.g. with light intensity, and the absence of a second equilibrium? It seems fruitless to extend such speculations further as long as so little remains known of the concrete mechanism of the oscillators. Nor, given current ignorance of their

detailed habits and niche-differences, can we pursue the possible functional meaning of these strong species differences.

IV. Functional Singleness; Structural Complexity

Throughout these papers we have used the term "pacemaker" to denote a functional entity without commitment to and without reference to any concrete mechanism. It is an undamped, self-sustaining oscillation in the control of activity and rest whose formal properties have functional meaning: they are clock-like. The recent work of Stephan and Zucker (1972); Moore (1974), and Rusak and Zucker (1975), leaves little doubt that our pacemaker is neural and probably located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Lesions of the SCN either destroy the rhythm completely or generate gross abnormalities, some of which are akin to the splitting phenomenon and others to the total aperiodicity exemplified in Figure 4 (Rusak and Zucker, personal communication).

A circadian pacemaker in the nervous system could in principle be a single cell, and there is no doubt that some single neurons can sustain a circadian oscillation (Strumwasser, 1965). But it is also likely that populations of tightly coupled cells function as a "single", "complex" pacemaker. That appears to be the case in the eye of *Aplysia* (Jacklet and Geronimo, 1971; but see Strumwasser, 1974). That mollusk has, therefore, as a minimum, two complex pacemakers, one in each eye. And there is at least one other in the parietovisceral ganglion (Strumwasser, 1974; see also Block and Lickey, 1973). Bilaterally symmetrical animals will often possess redundant pacemakers; that is established fact in cockroaches where there are pacemakers in both sides of the protocerebrum (probably in the optic lobes) each of which is adequate, itself, to drive a circadian rhythm of locomotion (Nishiitsutsjui-Uwo and Pittendrigh, 1968; Roberts, 1974; Caldarola and Pittendrigh, unpublished observations; Page, personal communication). Our point is simple: when we are dealing with the nervous system – indeed with multicellulars in general – we cannot assume that functional "singleness" implies anatomical, or structural, singleness. It remains to be seen whether the components of our complex pacemaker (functionally a single entity) can be traced to structural, anatomical components in the hypothalamus; and whether, indeed, each component (M and E) is itself not a population of tightly coupled cells. The behavior of the aperiodic hamster in Figure 4, characterized by the gradual "nucleation" of activity bursts is probably best regarded as the gradual return of synchrony (by mutual coupling) of a much larger number of constituent components (cells) than the two, grosser, subsets we have postulated as M and E (Pittendrigh, 1974).

V. Pacemaker Function: Time of Day and Time of Year

There are many properties that circadian pacemakers share with all other self-sustaining oscillators. For that very reason they merit not particular attention in

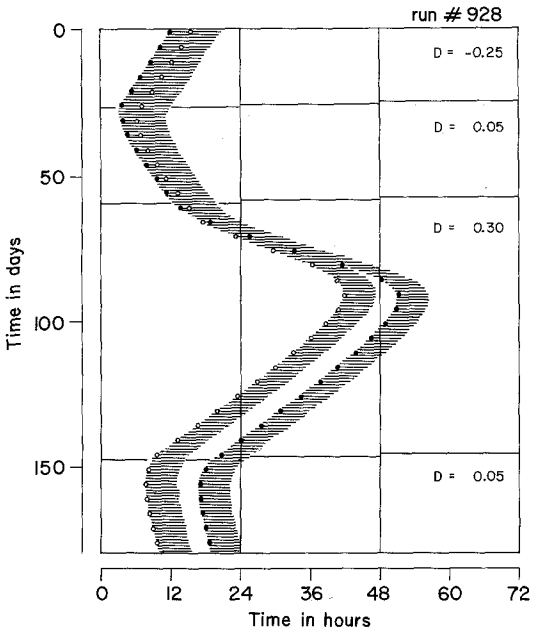

Fig. 10. Computer simulation of splitting in a two-oscillator model. For details of the algorithm see Daan and Berde (in preparation). Dots mark one of every five events in oscillator E; circles mark one of every five events in oscillator M. Each event is assumed to trigger five hours of "activity" (horizontal lines). Time is dimensioned to match the usual format of circadian actograms. For clarity, the "actogram" is "triple plotted": D-values indicate detuning of the two oscillators ($D = \tau_E - \tau_M$) in h, and increasing D is assumed to simulate increasing light intensity. The simulation shows: (i) increasing τ and decreasing α when D is raised from -0.25 to 0.05 (Aschoff's rule for nocturnal animals), including (ii) initial after-effects of $D = -0.25$ after the transition; (iii) a further increase in τ when D is raised further to 0.30, followed by (iv) a seemingly spontaneous split into two components, which is accompanied by (v) a decrease in τ; (vi) hysteresis: the split condition remains when D is lowered again to 0.05, where also the unsplit condition was stable; and (vii) different dependence of τ on D (light intensity) in the split and unsplit condition (from Daan and Berde, in preparation)

developing a model for the circadian case, even if, because understood, they yield to elegantly explicit mathematical treatment. It is the *uncommon behaviors* of circadian pacemakers that should be addressed in modelling if the canons of adequacy and utility are to be taken seriously.

The present model is loosely formulated but its essential propositions promise explanation of many circadian phenomena that no other model can handle, and no other oscillator we know of displays. Moreover these essential features have been found in a fully defined system of two mutually coupled oscillators (Daan and Berde, in preparation). Computer simulations show that the principal intuitive element is valid and has the expected consequences: the relative strength of the two oscillators' interactions is, as postulated here, dependent on their mutual phase-relations (ψ_{EM}). That dependence generates the after-effects and hysteresis we have stated it should; and the system displays some of the splitting behavior of the intuitive model including the change of τ that accompanies splitting (Fig. 10).

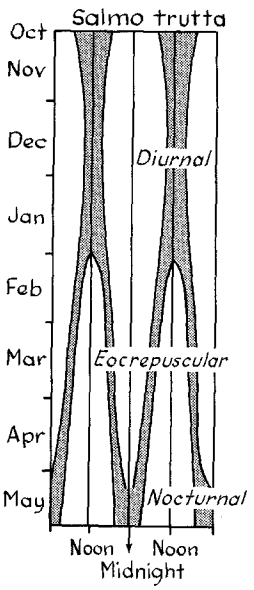

Fig. 11. The annual cycle of change in the daily activity pattern of young trout *Salmo trutta* (redrawn from Eriksson, 1973). The wide band of diurnal activity seen in the winter splits into two components in the spring as the photoperiod lengthens. One component follows sunrise, the other follows sunset. They re-fuse into a single nocturnal band in mid-summer when the arctic photoperiod increases toward 24 h

Our immediate interests are however in the analysis of functional organization and the model seems specially relevant to several problems in circadian physiology that derive from the annual cycle of change in the pattern of the day. One of these we have already addressed (Pittendrigh and Daan, 1976b): how is the phase-relation of pacemaker and external world conserved in the face of change in photoperiod? That question was found to have no unique answer: the appropriate strategy in adapting pacemaker properties (τ, PRC) was different for nocturnal and diurnal forms. Our previous discussion noted a different but related issue: there is no constant daily pattern throughout the year which any fixed innate program can match. Here the issue is not so much the simple conservation of activity onset to sunrise or sunset but moulding the time course of activity, as one aspect of behavioral and physiological functioning, to a constantly changing external pattern of day and night.

There are several aspects of this seasonal change that our complex pacemaker seems well equipped to handle. The classification nocturnal vs. diurnal is itself a simplification. Many animals, as different as insects and trout (Eriksson, 1973) show a dawn-dusk bimodality of activity (eo-crepuscular in the ecological jargon) which undergoes seasonal compression and decompression just as α in nocturnal rodents is compressed and decompressed by changing photoperiod (Fig. 1). The inadequacy of a single program may be met by entrusting the timing of separate parts to the two oscillators (M and E) comprising the complex-pacemaker and thereby timing activity to propitious conditions (temperature, humidity, light intensity) that will generally be bimodally distributed, even if asymmetrically, around the middle of the day. Eriksson's (1973) striking records of trout and salmon behavior throughout the year at the arctic circle are clearly tractable to our model as he himself notes (Fig. 11). The range of possible phase relationships between the two oscillators is expected to be adjusted to the ecological demands

of the species. Nearly complete locking onto dawn and dusk, as Eriksson's trout do, seems only possible if E and M are but loosely coupled to each other. Increasing tightness of coupling and homeostatic control may lead to a reduction of the variation of α with varying photoperiod, and among nine species of mammals and birds studied year-round at the arctic circle the activity time of hamsters was indeed the least affected by the short winter days and midsummernight sun (Daan and Aschoff, 1975).

There are some programs (for reproductive activity, hibernation, diapause, migration) for which a limited season is uniquely appropriate. Their initiation is very commonly triggered by photoperiod duration which is a reliable marker of season. Not long after Garner and Allard's (1923) discovery of "photoperiodic induction", Bünning (1936) published a profound intuitive hypothesis that what we would now call a circadian pacemaker was responsible for measuring the duration of the photoperiod. Bünning's classic paper added to this general proposition, itself now fully validated, a more specific formulation of how the pacemaker did so. In referring to "Bünning's Hypothesis" the literature often fails to distinguish this specific model of how the pacemaker does its job from the more general, and important, proposition that it does so *somehow* (cf. Pittendrigh and Minis, 1964, 1972).

Selection pressure to recognize the time of year is clearly strong and widespread outside the tropics. Circadian phenomena are not only complex but concretely very different in plants, insects and vertebrates. The empirical generalization, now established, if not without exception (Lees, 1971), that recognition of season nearly always has a basis in circadian physiology clearly provides – of itself – no assurance of a common mechanism. It seems almost certain that here, especially, evolutionary convergence is responsible for much of the similarity we find among plants, insects, vertebrates in the photoperiodic induction effected by exotic, experimental light cycles. The only conclusion warranted by current knowledge is that induction occurs in a limited array of entrained steady-states of the circadian system. There is a diversity of ways – even formally – in which entrainment phenomena could account for that generalization (Pittendrigh, 1972).

In briefly discussing one of those ways we do not imply it is the only mechanism of photoperiodic time-measurement. We expect diversity. But the type of complexity of pacemaker structure proposed in this paper has direct relevance to one model of photoperiodic induction which continues to receive inadequate attention, perhaps because the validity of Bünning's general proposition of circadian involvement is taken to imply the validity of his specific model ("external coincidence", see below).

In his 1936 paper Bünning envisaged photoperiodic induction as dependent on the coincidence of light and a critical (inducible) phase in the organism's circadian cycle of metabolic change. An entirely different approach, developed independently by Pittendrigh (1960, 1972, 1974) and Tyschenko (1966) bases recognition of a specific photoperiod on the phase-relationship it will generate between oscillations – all internal to the organism – coupled separately to sunrise and sunset. Here again the mechanism is essentially a coincidence-device; induction occurs only when, in a limited array of photoperiods, critical phases of constituent oscillators coincide, facilitating reactions that culminate in induction.

The distinction between this model and Bünning's is summarized by their designation as "internal" and "external" coincidence models (Pittendrigh, 1972).

The splitting phenomena and related behaviors summarized in these papers provide the first tangible evidence that circadian phenomena in animals do in fact involve oscillations which may be separately coupled to sunrise and sunset, and thereby enhance the plausibility that internal coincidence underlies at least some cases of photoperiodic induction.

The internal coincidence approach merits more attention for several reasons which our data make clear. Elliott (1974) has reported beautifully clear experiments on the photoperiodic control of testicular size in golden hamsters. Their testes grow in long days and regress when daylength is shortened. A one hour photoperiod every 24 h totally fails to induce gonadal growth, but when T, the period of the light cycle, is changed to 23.5 or 24.5 h, growth is promptly initiated by the one-hour light pulse driving the system. This type of experiment was initially designed by Pittendrigh and Minis (1964) in the hope of testing the "external coincidence" model. Clearly, if a critical photoinducible phase (ϕ_i) occurs near the end or the beginning of the subjective night it will be coincident with light only in some (long) photoperiods when $T=24$. But that coincidence should be effected, even using very short pulses if T is placed sufficiently far from τ to make the pulse coincident with ϕ_i in steady state. Elliott's results are certainly open to such interpretation in terms of external coincidence. But they are as readily explained by the change among internal oscillations which we propose is reflected in that compression of α at T 23.5 and T 24.5 which we know to occur.

The equivocal nature of these facts in attempting to discriminate between internal and external coincidence may well prove inherent in all purely formal analyses of photoperiodic induction. A major obstacle is that we have no reason to assume with confidence that one and the same circadian pacemaker is involved in the control of locomotion and gonadal growth. But it is not implausible, since in the female hamster estrus cycle and locomotor activity are intrinsically coupled, if not driven by the same pacemaker (Alleva *et al.*, 1971). The new evidence that the pacemaker for activity cycles in hamsters is in the suprachiasmatic nucleus (SCN) of the hypothalamus (Rusak and Zucker, 1975) is compatible with that proposition at least to the extent that the SCN is also known to be involved in the control of the gonadal state (Brown-Grant, personal communication). But proof of identity is still lacking. There is no doubt that progress depends largely on clarifying this issue and more generally in relating the formalism of circadian phenomena to the concrete neuroanatomy and physiology of the central nervous system.

Uncertainty that one and the same circadian pacemaker controls activity cycles and gonadal growth reduces the force of our finding no association between splitting of the activity pacemaker and testicular regression. Two groups of hamsters with fully developed testes were maintained in LL for three months while their activity cycles were monitored. Testes size was assayed by palpation in 10 split and 10 unsplit animals. Had the gonads of split animals declined in spite of the continuous illumination the correlation with change in the phase-relations of observable circadian oscillations would have given strong evidence for an internal coincidence mechanism. The negative result remains equivocal.

On the other hand there are two important observations that seem to elude explanation in any other way and their force is unimpaired by ignorance of concrete detail. Prompted by the fact that circadian oscillations in poikilotherms can be as readily entrained by temperature cycles as by light, Pittendrigh (1972) pointed out that when internal coincidence does underlie "photoperiodic" induction in poikilotherms, light should be entirely dispensable in the time-measurement: it should be possible to create the necessary phase-relations between internal oscillators by changing "thermoperiod". Saunders (1973) has pursued this proposal and found that in the wasp *Nasonia,* diapause can be as rigorously controlled by change in thermoperiod, in total darkness, as by the normal change in photoperiod. External coincidence of light and some ϕ_i is here clearly excluded. There is a second observation which defies easy explanation by external coincidence but is readily understood in terms of internal coincidence and the phenomenon of pacemaker after-effects. Birds *(Junco hyemalis)* maintained on an inductive photoperiod for only 8 days continue to maintain testicular growth when placed in constant darkness although transfer to short days immeddiately arrests that growth (Wolfson, 1966, 1970). D. S. Farner (personal communication) has found the same result using *Passer domesticus.* We see here the effect of a long photoperiod establishing a set of phase relations between constituent oscillators that induce gonadal growth, and the inertia of the system, reflected in after-effects, retaining that inductive state in constant darkness. Short days, on the other hand, have an immediate positive action in destroying the inductive phase-relation between morning and evening oscillators.

Some form of the internal coincidence model seems necessary to explain the results of Meier et al. (1971) showing that gonadal growth in sparrows is dependent on the circadian phase relationship between hormonal rhythms. Further analysis of the mechanism of photoperiodic time measurement is now clearly far more dependent on the development of an experimental system in which the concrete physiological controls of gonadal cycles are becoming better known, and the physical location of the pacemaker reasonably well established. It is doubtful if the strictly formal analyses of the recent past can go beyond establishing – as they already have – the validity of Bünning's general proposition. But the formal aspects of how circadian pacemakers are entrained, as well as their behaviour while freerunning, nevertheless provide valuable constraints and guides in proceeding to neurophysiological and endocrinological detail. Whatever the concrete detail proves to be, we strongly suspect that especially in higher animals some form of internal coincidence will be involved in photoperiodic induction.

We close by stressing that the two-oscillator structure we propose for the pacemaker offers a basis for accommodation to seasonal change in two quite different respects. On the one hand it meets the challenge of seasonal change in the *pattern of the external day,* which no single program – in the hands of a single pacemaker – seems adequate to cope with. Its two components, responding differentially to sunrise and sunset, would make the pacemaker an adequate clock for every differently patterned day in the year. On the other hand that same complexity is a potential sensor for the *time of year.* It is a clock for all seasons.

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